Elevated serum melatonin is associated with the nocturnal worsening of asthma

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Background: Increased airway inflammation at night contributes to the nocturnal worsening of asthma. In vitro studies have shown exogenous melatonin to be pro-inflammatory in asthma, but it is unknown whether endogenous melatonin levels are a controller of airway inflammation in nocturnal asthma.
Objective: Our aim was to determine 24-hour patterns of serum melatonin and their relationship to overnight decline in physiology in subjects with nocturnal asthma, non-nocturnal asthma, and in healthy controls.
Methods: Observational study of pulmonary physiology and melatonin levels in patients with nocturnal asthma (n = 7), non-nocturnal asthma (n = 13), and healthy controls (n = 11). Subjects maintained a constant sleep–wake regimen for 7 days. On day 8, serum melatonin was measured every 2 hours by radioimmunoassay and analyzed by cosinor modeling. The correlation between serum melatonin levels and overnight change in spirometry was evaluated by Spearman's rank correlation analysis.
Results: In subjects with nocturnal asthma, peak melatonin levels were significantly elevated compared with healthy controls (67.6 ± 5.0 pg/mL versus 53.5 ± 4.0 pg/mL, *P* = .03). Melatonin acrophase was delayed in nocturnal asthma (02:54 versus 01:58 in healthy controls, *P* = .003, and 02:15 in non-nocturnal asthma, *P* = .01). In subjects with nocturnal asthma, increasing melatonin levels were significantly and inversely correlated with overnight change in FEV_1 (*r* = –.79, *P* = .04), a relationship that was not observed in non-nocturnal asthma or healthy controls.
Conclusions: Nocturnal asthma is associated with elevation and phase delay of peak serum melatonin levels. Elevated melatonin levels might contribute to the pathogenesis of nocturnal asthma. (J Allergy Clin Immunol 2003;112:513-7.)

Key words: *Melatonin, circadian rhythm, inflammation*

Nocturnal asthma is an asthma phenotype in which airway inflammation is increased during the hours of sleep.[1] Nocturnal increases in airway inflammation are associated with increased airway hyperresponsiveness[2] and worsened expiratory airflow limitation,[3] which combine to cause physiologic worsening at night and disruptive nocturnal symptoms such as cough and dyspnea. These nocturnal symptoms are considered a clinical marker of asthma severity and are an important factor in determining appropriate asthma pharmacotherapy.[4] Although a number of factors including alveolar inflammation,[5] peripheral blood eosinophil number and function,[6] and glucocorticoid and β-adrenergic receptor affinity or activity[7,8] have been implicated in the pathogenesis of nocturnal asthma, the mechanisms regulating this asthma phenotype remain unclear.

Abbreviation used
AUC: Area under the curve

Melatonin (*N*-acetyl-5-methoxytryptamine) is the principal hormone product of the pineal gland. Its serum levels fluctuate across the 24-hour circadian period, and it is a key regulator of circadian rhythms in humans.[9] Melatonin is immunomodulatory and can induce human lymphocytes and monocytes to synthesize cytokines including IL-2, IL-6, and IL-12.[10,11] Endogenous melatonin might affect asthma severity, because it has been shown to enhance allergic airway inflammation[12] and airway smooth muscle tone in animal models.[13,14] In ovalbumin-sensitized mice, melatonin enhances proliferation of antigen-specific $CD4^+$ T cells and induces a T_H2-like immune response as manifested by secretion of IL-4 and down-regulation of IL-2 and interferon-γ.[15] In human subjects with asthma, melatonin is pro-inflammatory, increasing PBMC production of IL-1, IL-6, and TNF-α.[16] Melatonin's immunomodulatory effect also varies with asthma clinical phenotype and clock time, with differential effects at 04:00 and 16:00 in subjects with nocturnal asthma versus non-nocturnal asthma.[16] This pro-inflammatory effect might have clinical relevance, because it has been suggested that millions of Americans use over-the-counter melatonin as a sleep aid.[17] Given the pro-inflammatory effects of melatonin in asthma, its use might be deleterious to patients with asthma.

To test the hypothesis that circadian variations in melatonin are correlated with the severity of nocturnal asthma, we performed an observational study of 24-hour melatonin levels in patients with nocturnal asthma, non-nocturnal asthma, and healthy controls.

METHODS

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

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TABLE I. Subject characteristics

	Nocturnal asthma (n = 7)	Non-nocturnal asthma (n = 13)	Controls (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*
Morning FEV_1 (L, mean ± SEM)	2.40 ± 0.34	3.32 ± 0.25	3.89 ± 0.27	.008*
Overnight % change in FEV_1 (mean ± SEM)	–18.8 ± 2.3	–5.1 ± 2.1	+1.5 ± 2.3	<.0001*

*One-way ANOVA.
†Fisher exact test.

Classification of subjects

Nonsmoking adult healthy controls, subjects with nocturnal asthma, and subjects with non-nocturnal asthma were eligible for this study. Asthma was defined by (1) a suggestive clinical history and either (2) airway hyperresponsiveness documented by evidence of a ≥ 20% decline in FEV_1 in response to inhalation of methacholine at concentrations ≤ 8 mg/mL, or (3) evidence of expiratory airflow limitation (FEV_1 55%–85% of predicted) that was bronchodilator-responsive (documented by an increase in FEV_1 of ≥200 mL and 12% in response to 180 μg albuterol aerosol). Subjects with nocturnal asthma met the preceding criteria and demonstrated an overnight fall in the peak expiratory flow rate of ≥20% on 4 of 7 nights at home and an in-laboratory overnight fall in FEV_1 of ≥15%.

Subjects were excluded if they had a lung disease other than asthma, other medical illness, had used systemic or inhaled corticosteroids within 12 weeks, or had used leukotriene modifiers within 12 weeks of study enrollment. The use of antihistamines was allowed.

Sleep-wake routine

Subjects maintained a regular sleep–wake routine for 1 week before evaluation, sleeping at 22:00 and awakening at 06:00 for 7 sequential days. No controls were placed on ambient light, bed position, or caloric intake. Adherence to the sleep–wake routine was evaluated 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 on awakening.

Serum melatonin

Serum melatonin was assayed by radioimmunoassay (Yerkes National Primate Research Center, Atlanta, Ga).

Statistical analysis

Between-group differences in individual hormone levels were analyzed in 2 ways: (1) cosinor analysis of 24-hour circadian rhythm,[18] and (2) comparison of area under the 24-hour cosinor curve (AUC). We developed a cosinor model to analyze the 24-hour circadian pattern of melatonin. This circadian rhythm was fitted using a maximum function of a simple cosinor model with a period of 24 hours by means of a nonlinear mixed-effects model method:

$$\text{melatonin}_k = \max[0, \mu_k + \alpha_k \cos(\frac{2\pi \times \text{time}}{24} - \delta_k)]$$

This model was fitted to 3 groups (k = healthy controls, subjects with nocturnal asthma, subjects with non-nocturnal asthma) simultaneously to find vertical shift (μ), amplitude (α), and phase (δ) for each group. Maximum melatonin level, peak, and onset times were calculated using the parameter estimates and compared among the 3 groups.

All parameter estimations and comparisons were performed using SAS procedure NLMIXED (SAS version 8.02, SAS Institute, Cary, NC).[19] The AUC for melatonin was calculated using subject-specific predictions of the preceding cosinor models and compared among the 3 groups by means of a 1-way ANOVA. Spearman's rank correlation analysis was used to evaluate the relationship between observed peak melatonin level and overnight change in FEV_1. A 2-tailed P value < .05 was considered significant.

RESULTS

Subject characteristics

Seven subjects with nocturnal asthma, 13 subjects with non-nocturnal asthma, and 11 healthy controls were evaluated. Demographic and spirometric characteristics of subjects are presented in Table I. Bedtime FEV_1 did not differ significantly among groups (Table I), and subjects with nocturnal asthma had significantly lower morning FEV_1 (2.40 ± 0.34 L) and greater overnight decline in FEV_1 (–18.8% ± 2.3%) versus patients with non-nocturnal asthma and healthy controls (Table I).

Analysis of serum melatonin

Peak melatonin levels were significantly higher in subjects with nocturnal asthma than in healthy controls (67.6 ± 5.0 versus 53.5 ± 4.0 pg/mL, P = .03). Calculated area under the 24-hour melatonin curve did not differ between the 3 groups (Table II) caused in large part by the absence of between-group differences between 08:00 and 20:00 because of unmeasurable melatonin levels. In nocturnal asthma, melatonin acrophase was delayed when compared with non-nocturnal asthma (39 minutes, P = .003) and controls (56 minutes, P = .01, Table II). The modeled 24-hour circadian rhythm of melatonin is shown in Fig 1.

Correlation of melatonin with overnight fall in FEV_1

Peak measured serum melatonin level was significantly and inversely correlated with overnight change in FEV_1 in subjects with nocturnal asthma (Fig 2, r = –.79, P = .036), but not in subjects with non-nocturnal asthma (Fig 2, r = –.24, P = .44) or healthy controls (Fig 2, r = .43, P = .19).

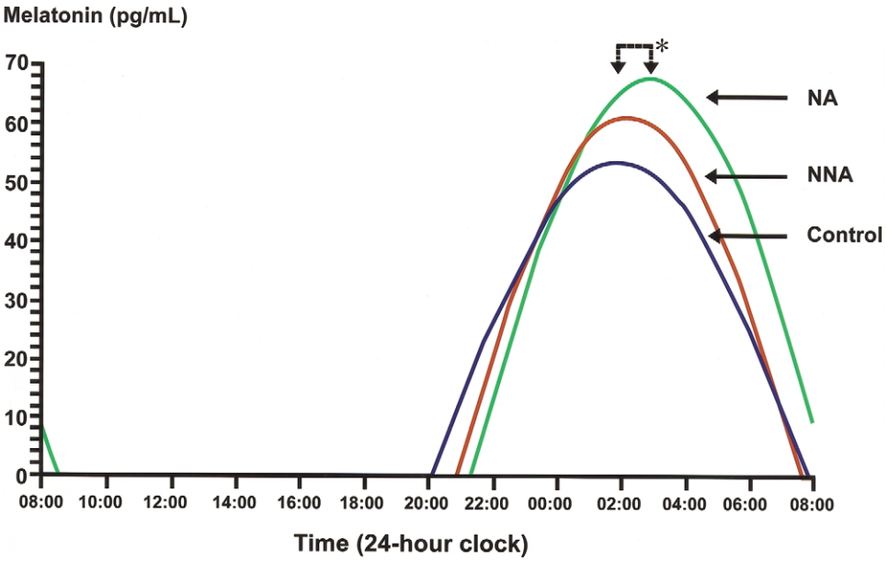

FIG 1. Twenty-four–hour pattern of melatonin levels in subjects with nocturnal asthma *(green)*, non-nocturnal asthma *(red)*, and in healthy controls *(blue)*. Paired *horizontal arrows* indicate a phase delay in peak melatonin time in nocturnal asthma. *Nocturnal asthma > controls, $P < .05$.

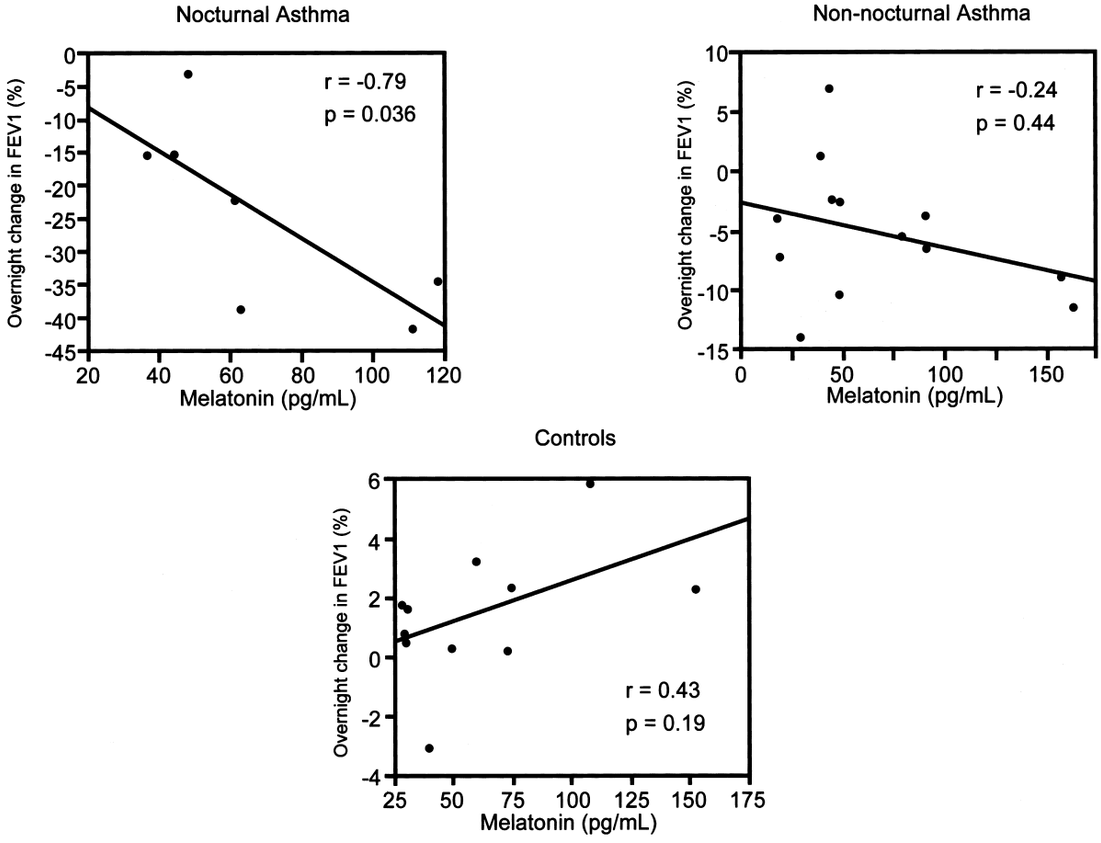

FIG 2. Correlation between peak serum melatonin level and overnight change in FEV_1 in subjects with nocturnal asthma, non-nocturnal asthma, and in healthy controls.

TABLE II. Peak hormone levels (mean ± SEM), area under the 24-hour cosinor curve and time of peak and trough melatonin levels (24-hour clock time) in subjects with nocturnal asthma, non-nocturnal asthma, and healthy controls

	Nocturnal asthma (n = 7)	Non-nocturnal asthma (n = 13)	Controls (n = 11)
Melatonin (pg/mL)	67.6 ± 5.0*	61.1 ± 3.6	53.5 ± 4.0
Melatonin AUC (pg × hr/mL)	541.7 ± 72.2	509.0 ± 61.3	480.6 ± 66.4
Melatonin peak time	02:54†	02:15	01:58
Melatonin onset time	21:21	20:55	20:09

*P = .034 for nocturnal asthma versus control, P = .16 for nocturnal asthma versus non-nocturnal asthma, and †P = .003 for comparison with controls and P = .011 for comparison with non-nocturnal asthma.

DISCUSSION

These experiments demonstrate that endogenous melatonin levels are elevated in subjects with nocturnal asthma and that in this asthma phenotype alone melatonin levels are negatively correlated with overnight change in FEV_1. This suggests that the elevated melatonin levels observed in nocturnal asthma might affect the severity of airway inflammation in this group.

Melatonin is an in vitro immunomodulator in human asthma,[16] and our data suggest an in vivo effect in humans as well. Prior research has demonstrated that costimulation of PMBCs with melatonin is pro-inflammatory independent of asthma phenotype, resulting in increased PBMC production of IL-1, IL-6, and TNF-α at both 04:00 and 16:00 in subjects with nocturnal asthma, subjects with non-nocturnal asthma, and controls. Furthermore, unlike non-nocturnal asthma, PBMCs from subjects with nocturnal asthma were unable to mount an additional cytokine response to melatonin at 04:00, suggesting that maximal stimulation might already be occurring in vivo in the nocturnal asthma phenotype.[16] Our observation that melatonin levels are highest in patients with nocturnal asthma supports this conjecture.

Despite attempts to similarly entrain sleep–wake cycles in all 3 groups, melatonin acrophase was significantly delayed in those with nocturnal asthma. Whether this reflects a primary abnormality of melatonin circadian rhythm or is a secondary response to altered sleep patterns in nocturnal asthma is unknown. Phase delay of melatonin might also be a compensatory attempt to delay the time of maximal pro-inflammatory effect past that when airway inflammation approaches its maximal level.

Although between-group numeric differences in 24-hour melatonin AUC were observed, these differences did not achieve statistical significance. This likely reflects aspects of both study design and biology; a larger sample size would have increased the power to detect between-group differences, but the fact that melatonin levels are unmeasurable in all 3 groups for approximately 50% of the 24-hour period and differ primarily in their peak values (Fig 1) suggests that it is largely peak melatonin levels that drive differences in the AUC.

Certain methodologic features differentiate this experiment from prior research in this area. Subjects' sleep–wake cycles were standardized in an attempt to minimize any environmental cues that might affect nocturnal worsening of asthma. Although a formal "constant routine" protocol[20] would have more rigorously controlled these cues, these protocols differ so dramatically from typical human experience that the clinical relevance of our results would be difficult to interpret. Second, we sampled serum hormone levels at 2-hour intervals rather than relying on peak and trough levels alone or less-frequent measures. In addition, the cosinor analysis used to model these data allows comparisons at individual time points and also facilitates modeling of the 24-hour pattern of hormone flux, allowing comparison of peak and trough times and calculation of the AUC. This analytic technique is likely to more accurately reflect events over the entire 24-hour period and allows inferences to be made not from comparisons between individual time points or from 24-hour mean levels[21] but from modeled changes over the course of time.

The clinical implications of this research require further investigation. Systemic corticosteroid therapy in asthma has been reported to alter melatonin circadian rhythm,[22] and this might suggest a mechanism by which chronotherapeutic dosing of corticosteroids reduces airway inflammation in nocturnal asthma.[23] Furthermore, although exact figures on exogenous melatonin use are not readily available, it has been suggested that millions of Americans (approximately 6% of whom are likely to be asthmatic) use supplemental melatonin.[17] Given that melatonin enhances inflammation in asthma and that higher levels are associated with more severe physiologic impairment in nocturnal asthma, these data suggest that avoidance of exogenous melatonin supplementation by persons with asthma might be warranted until further research explains the clinical effect of melatonin in asthma.

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