Priority of Peak Circadian Variation of Bronchial Responsiveness to the Trough of Circadian Variation of Bronchial Caliber in Asthmatic Children*  
Shinya Kondo, M.D.; and Kiyoko Abe, M.D.

To study the temporal relation between the peak of circadian variation of bronchial responsiveness and the trough of circadian variation of bronchial caliber, we performed seven inhalation challenges with histamine at 4-h intervals in six stable asthmatic children aged eight to ten years. Bronchial responsiveness was expressed as PC20. Coefficient of variation of baseline FEV, within the study day was less than 7 percent in all. The trough of FEV, variation by cosinor analysis ranged from 02.50 to 11.99 h (mean, 05.66). All had both significant (p<0.05) or marginally significant (0.05<p<0.1) cosinoidal rhythm of PC20 and two or more doubling concentration differences between the highest and lowest PC20s. The trough of PC20 variation ranged from 16.32 to 02.57 h (mean, 2.30). There was a significant (p<0.05) difference between troughs of FEV, and PC20 variations. We conclude that the peak of circadian variation of bronchial responsiveness precedes the trough of circadian variation of bronchial caliber in asthmatic children. *(Chest 1991; 100:640-43)*

PC20 = provocative concentration of histamine causing a 20 percent fall in FEV,

Most severe bronchial obstruction usually occurs from midnight to early morning in asthmatic patients.1,2 Increase in nonspecific bronchial responsiveness is roughly considered as one of the participant factors that causes bronchial obstruction in nocturnal asthma. Van Alderen et al recently studied circadian variation of bronchial responsiveness in asthmatic children whose baseline FEV, levels were kept almost the same throughout the study. They expressed the degree of bronchial responsiveness as mean value of the group at absolute hours. In their study, the peak of circadian variation of bronchial responsiveness was at 4 AM, when bronchial obstruction usually becomes most severe. By contrast, several studies denied the direct relation between bronchial responsiveness and bronchial caliber.4-6 Also, in late asthmatic response after antigen inhalation, further increase in bronchial responsiveness preceded further obstruction in bronchial caliber.6,7 The temporal relation between the peak of circadian variation of bronchial responsiveness and the trough of circadian variation of bronchial caliber in nocturnal asthma may be directly studied in patients who have nocturnal bronchial obstruction. The amplitude of circadian variation of bronchial responsiveness in those patients may be high enough to make analysis clinically understandable. However, the difference in baseline bronchial caliber affects the measurements of bronchial responsiveness by a geometric effect. Therefore, we studied the relation in stable asthmatic children whose circadian variation of provocative concentration of histamine causing a 20 percent fall in FEV, (PC20) had both cosinoidal rhythm and appreciable amplitude.

**Patients and Methods**

**Patients**

The study group consisted of six asthmatic children in remission; four of them were boys. The mean age was nine years (range, eight to ten years) (Table 1). They were staying in a residential treatment center for at least eight months because of uncontrollable asthma at their home, and performed spirometry almost every morning. All had episodes of nocturnal asthma within the last three months. Coefficient of variation on seven consecutive morning FEV, levels before the beginning of the study was less than 5 percent in all. No child took oral or inhaled corticosteroids for at least a year before the study. Asthma symptoms were well controlled by regular sodium cromoglycate inhalation or oral ketotifen, and by oral salbutamol and aminophylline as required. None of them had respiratory infection for at least six weeks before the study.

They stayed indoors quietly except for a walk around the center throughout the study. Although they went to bed at 8:30 PM and got up at 6 AM, they could lie down at any time when they wanted.

**Table 1—Some Characteristics of the Patients**

<table>
<thead>
<tr>
<th>Patient No./ Age, yr/Sex</th>
<th>Height, cm</th>
<th>Eosinophil, per cu mm</th>
<th>Maintenance Medication</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/8/M</td>
<td>122</td>
<td>485</td>
<td>Cromoglycate</td>
</tr>
<tr>
<td>2/10/M</td>
<td>132</td>
<td>564</td>
<td>Ketotifen</td>
</tr>
<tr>
<td>3/8/M</td>
<td>123</td>
<td>781</td>
<td>Cromoglycate</td>
</tr>
<tr>
<td>4/9/F</td>
<td>135</td>
<td>512</td>
<td>Ketotifen</td>
</tr>
<tr>
<td>5/9/F</td>
<td>122</td>
<td>616</td>
<td>Cromoglycate</td>
</tr>
<tr>
<td>6/10/M</td>
<td>132</td>
<td>624</td>
<td>Ketotifen</td>
</tr>
</tbody>
</table>

*Circadian Variations in Bronchial Caliber of Asthmatic Children (Kondo, Abe)*
Although the other 14 patients were studied, they were excluded from the study because of significant variation in baseline FEV$_1$, nonsignificant amplitude, and nonsignificant fit of cosinusoidal model in PC20 variation.

Written consent was given by each parent. The study protocol was approved by the Clinical Research Committee, Children's Asthmatic Center, Kawasaki City Ida Hospital.

**Study Design**

The study was performed from July to October, 1988. Indoor temperature was kept 20°C to 25°C, and humidity was 69 to 87 percent. Treatment with all medications was withdrawn for at least 24 hours before the study. Seven histamine inhalation tests were performed repeatedly at 4-h intervals from 10 AM in the first study day to 10 AM in the second day. At 10 PM and 2 AM, the tests were performed after the patients became fully awake.

FEV$_1$ was expressed as FEV$_1$ percent pred based on the equation of Sumida$^2$ for Japanese schoolchildren, which is as follows: FEV$_1$ (ml) in boys: 34.82 × height (cm) - 2746; FEV$_1$ (ml) in girls: 34.42 × height (cm) - 2829.

**Histamine Inhalation Tests**

Histamine hydrochloride dilution in saline solution (range, 0.06 to 8 mg/ml) was used for bronchial provocation. After the baseline FEV$_1$, had been recorded with hot wire AS-1500 autospirometer (Minato Medical Science, Osaka, Japan), histamine inhalation tests were performed using two minutes' tidal breathing technique similar to that described by Cockcroft et al.$^3$ The dilution of histamine was nebulized with a glass jet nebulizer (Nishosuki, Nihon Shoji, Osaka, Japan) with an airflow of 8 L/min. The aerosol particle size by the nebulizer was 1 to 10 μm, and the nebulizer output of distilled water was 0.20 ml/min. The patients inhaled aerosol through a mouthpiece. FEV$_1$ was measured at 30 and 90 s after the end of each inhalation. The best of the two values was taken for the evaluation at each step. The procedure was continued with doubling concentrations of histamine until there was a 20 percent fall in FEV$_1$ from the baseline value or when the maximum concentration was given. Bronchial responsiveness was expressed as PC20. PC20 was directly calculated by linear interpolation between the last two points on either side of the line for 20 percent fall.$^4$ The test is repeatable within one doubling concentration difference within a patient, and two or more doubling concentration differences are regarded as significant in our center. When PC20 at 10 AM on the second day was within one doubling concentration difference of that in the first day with significant or marginally significant cosinusoidal rhythm and there were two or more doubling concentration differences between the highest and lowest PC20s, the circadian variation of PC20 was regarded to have the circadian rhythm. The same nebulizer was used for the same patient throughout the study.

**Analysis**

Six measurements of FEV$_1$ and PC20, excluding values at 10 AM from the first study day, were used for cosinor analysis and calculation of mean, SD, and coefficient of variation.

Cosinor analysis introduced by Halberg et al.$^5$ was performed to evaluate the rhythm and the trough hour of circadian variation of FEV$_1$, for each patient. When coefficient of variation of six baseline FEV$_1$ determinations within a day was less than 7 percent, the difference was considered nonsignificant.$^6$ Circadian variation of PC20 was evaluated by both cosinor analysis and the difference between the highest and lowest PC20s. Cosinor analysis of PC20 was performed after the logarithmic translation of the data. Cosinusoidal rhythm was found to be significant when the amplitude of the curve differed from zero with p<0.05 and marginally significant with 0.05<p<0.1. The values of PC20 more than 8 mg/ml were excluded from the analysis. Sign test was used for evaluating whether the PC20 changes precede the FEV$_1$ changes, and statistical significance was accepted at p<0.05. Statistical analysis of the data was carried out (using the SAS PROC NLIN).

**RESULTS**

Information describing each patient involved in the study is given in Table 2 and Figure 1.

In each patient, the difference between baseline FEV$_1$, at 10 AM on the first and second day was within 10 percent of the former value. Cosinusoidal rhythm was significant in two patients (patients 3 and 6) but nonsignificant in the remaining four patients. Mean of six baseline FEV$_1$ tests in each patient ranged from 76.5 to 110.7 percent predicted. The trough of circadian variation of FEV$_1$ ranged from 02.50 to 11.99 h (mean, 05.66; SD, 3.38). Coefficient of variation of six baseline FEV$_1$ determinations was less than 7 percent in each patient.

The final inhaled histamine concentration at 10 AM on the second day was the same as that on the first day in each patient. Three patients (patients 2, 4, and 5) had significant, and three patients (patients 1, 3, and 6) had marginally significant, cosinusoidal rhythms of PC20. Geometric mean of six PC20s in each patient ranged from 0.11 to 1.95 mg/ml. The trough of circadian variation of PC20 ranged from 16.32 to 02.87 h (mean, 22.30; SD, 4.11). All had two or more doubling concentration differences between the highest and lowest PC20s. By comparing the trough hours with respect to PC20 and FEV$_1$, in each patient, it was shown that the PC20 changes precede the FEV$_1$ changes (p = 0.014).

**DISCUSSION**

The present study suggested that the peak of circadian variation of bronchial responsiveness does not coincide with the trough of circadian variation of bronchial caliber. In six patients whose circadian variation of PC20 had both significant or marginally significant cosinusoidal rhythm and significant differ-

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>PC20</th>
<th>FEV$_1$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mesor, log$_{10}$mg/ml</td>
<td>Trough, hour</td>
</tr>
<tr>
<td>1</td>
<td>1.01</td>
<td>02.26</td>
</tr>
<tr>
<td>2</td>
<td>1.01</td>
<td>02.87</td>
</tr>
<tr>
<td>3</td>
<td>0.37</td>
<td>23.86</td>
</tr>
<tr>
<td>4</td>
<td>0.11</td>
<td>20.93</td>
</tr>
<tr>
<td>5</td>
<td>0.65</td>
<td>19.53</td>
</tr>
<tr>
<td>6</td>
<td>1.95</td>
<td>16.32</td>
</tr>
</tbody>
</table>
ence between the highest and lowest PC20s, the trough of circadian variation of PC20 ranged from 16.3 to 02.87 h. By contrast, the trough of circadian variation of FEV, ranged from 2:30 AM to just before noon. In addition, it is well known that the most severe bronchial obstruction in nocturnal asthma usually occurs from midnight to early morning. These suggest that circadian rhythm of bronchial responsiveness may differ from that of bronchial caliber, and that the peak of circadian variation of bronchial responsiveness may precede the trough of bronchial caliber in asthmatic children.

De Vries et al. suggested that increase in bronchial responsiveness preceded decrease in bronchial caliber in nocturnal asthma in 11 adult asthmatics. However, their results are difficult to interpret because of the significant variation in baseline FEV, Rachiele et al. studied circadian variation of PC20 in 15 adult asthmatics. The trough of variation was distributed widely within a day in their study. However, more than half of their patients already had bronchial obstruction before the tests, and many of the amplitudes of variation were within a reproducible range of the test. Van Aalderen et al. reported circadian variation of PC20 in nine asthmatic children whose baseline FEV, were almost unchanged throughout the study day. In their study, the trough of PC20 variation by visual inspection was 4 AM, when most severe bronchial obstruction usually occurs in nocturnal asthma. The finding of the present study was inconsistent with their finding. However, the aim of their study was not to show the temporal difference between bronchial responsiveness and bronchial caliber. They showed the values of PC20 as mean of the group at absolute hours.

Bronchial inflammation can explain further increase in bronchial responsiveness and subsequent bronchial obstruction, but it can hardly explain alternate increase and decrease in bronchial responsiveness with 24-hour periodicity. There are a few possible explanations for the temporal discrepancy between the peak of circadian variation of bronchial responsiveness and the trough of circadian variation of bronchial caliber in asthmatic children, although the exact mechanism is uncertain. First, earlier studies demonstrated that the troughs of circadian variation of plasma epinephrine and cortisol, protective factors against bronchial inflammation, were in the early morning. So, increase in bronchial responsiveness precedes not only bronchial obstruction but also the troughs of circadian variation of plasma epinephrine and cortisol. Increase in bronchial responsiveness before the drop of protective factors against bronchial inflammation suggests that bronchial responsiveness may have an independent biologic rhythm of its own. Second, bronchial obstruction in asthma is the result of excessive response to a wide variety of immunologic, pharmacologic, and physical stimuli. PC20 in the present study represented only a part of the bronchial responsiveness measured by a transient bronchial
smooth muscle contraction induced by histamine inhalation. Only the rhythm of bronchial responsiveness to histamine among total bronchial responsiveness may differ from that of bronchial caliber. Third, minimum bronchial smooth muscle contraction may occur soon after increase in bronchial responsiveness. In addition to the muscle contraction, mucosal edema following bronchial smooth muscle contraction may develop slowly. The mucosal edema may be responsible for early morning bronchial obstruction, which may be improved by increased secretions of epinephrine and cortisol in the early morning.

The question has been raised as to whether it is appropriate to analyze low amplitude data by cosinor analysis, because the results might be inconsistent with clinical observations in asthmatics. In all our patients, the difference between the highest and lowest PC20s within a day exceeded the reproducible range of histamine inhalation test. However, the change of FEV1 within a day was not significant because of the necessity of stable baseline FEV1 for histamine inhalation tests. Most troughs of circadian variation of FEV1 by cosinor analysis, nevertheless, did not differ from clinical findings recognized in nocturnal asthma. Also, circadian variations of PC20 and FEV1 in asthmatics do not necessarily fit cosinusaloidal rhythm. Several endogenous and exogenous factors, with or without rhythm, have synergistic effects on circadian variations of PC20 and FEV1, and therefore, the variations may not be pure. For this reason, we calculated the trough of circadian variation of PC20 in patients with not only significant but also marginally significant cosinusaloidal rhythms of PC20.

It is desirable for the investigation of temporal difference between PC20 and FEV1, and for cosinor analysis to measure PC20 and FEV1 as frequently as possible. However, the earlier studies suggested that repeated histamine inhalations might cause tachyphylaxis to histamine in asthmatic patients. By contrast, the other studies suggested the repeatability of PC20 or even cumulative effect of histamine after appreciable bronchoconstriction in their repeatability studies with histamine. In all our patients, the final inhaled histamine concentration at 10 AM on the second day was the same as that at 10 AM on the first day after getting through fall and rise within a day. This may imply that the effect of histamine inhalations at 4 h intervals on measurement of PC20 was minimum in the present study.

ACKNOWLEDGMENT: We sincerely thank the nurses of Children's Asthmatic Center, Kawasaki City Ida Hospital, for technical assistance, and Mr. Hileki Origasa for statistical analysis.

REFERENCES
1 Hetzel MR, Clark TJJH. Comparison of normal and asthmatic circadian rhythms in peak expiratory flow rate. Thorax 1980; 35:732-38
5 Galvez RA, McLaughlin FJ, Levison H. The relationship between airway obstruction and bronchial hyperreactivity in childhood asthma. Ann Allergy 1987; 58:45-7
7 Cockcroft DW, Murdock KY. Changes in bronchial responsiveness to histamine at intervals after allergen challenge. Thorax 1987; 42:302-08
8 Sumida N. Normal values for pulmonary function tests in schoolchildren. Pediatr Jpn 1976; 17:431-32
13 De Vries K, Goei JT, Booy-Noord H, Orie NGM. Changes during 24 hours in the lung function and histamine hyperreactivity of the bronchial tree in asthmatic and bronchitic patients. Int Arch Allergy 1962; 20:35-101
20 Barnes PJ. Inflammatory mechanisms and nocturnal asthma. Am J Med 1988; 85(suppl 1B):64-9
21 Schoeffel RE, Anderson SD, Gillam I, Lindsay DA. Multiple exercise and histamine challenge in asthmatic children. Thorax 1986; 35:164-70