

Circadian variation of skin reactivity and allergy skin tests

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Previous investigations of the circadian variation in skin reactivity suggested that results of skin tests obtained in the afternoon could vary from the results obtained in the early morning and therefore could result in a differing assessment of patient sensitivity. To determine whether this was a practical concern in the normal clinical setting, we studied 20 adults and 20 children who had skin prick tests positive (3+ or more) to short ragweed. These patients were skin tested in duplicate at 8 AM and at 4 PM with fivefold serial dilutions of short ragweed extracts (1:20 to 1:12,500, wt/vol) and of histamine hydrochloride (10 to 0.016 mg/ml). Areas of wheal and flare were recorded and measured by computed planimetry. In addition, results were also read according to a conventional scoring system. Mean wheal and erythema areas with ragweed and histamine at each dilution were compared between morning and their corresponding evening values. Although there was a trend for the morning means to be larger than evening means, no significant differences between the two sessions were observed at any dilution. Mean morning skin index scores, as calculated from the combined mean wheal and erythema areas, were larger than mean evening scores for ragweed and histamine, but the differences were not of a degree to be clinically important. This observation was also true for conventional scores. Comparing the results from the two groups of children who had their first set of skin tests performed either in the morning or afternoon session indicated that there was no evidence of a refractory state of the skin during the second test sessions. We conclude that circadian variation of skin reactivity within the customary clinic hours is minimal and should not affect the clinical interpretation of skin tests whether performed in the morning or the afternoon. (J ALLERGY CLIN IMMUNOL 1989;83:1101-6.)

Circadian variations in body responses (chronobiology) exist in both health and disease. Nocturnal asthma, an exaggeration in the circadian variation in the airway caliber, is among the better known examples of these phenomena. Circadian variation of skin reactivity also occurs but has received little attention from practicing allergists, despite its potential influence on clinical diagnosis in allergy. In 1965, Reinberg et al.¹ demonstrated that skin reactivity to histamine and to compound 48/80 was maximal between 7 to 11 PM and was at its nadir at 7 AM. Since the skin response to histamine paralleled that to compound 48/80 during the entire period of the study, the origin of the circadian variation must have been variation in the skin response to mediators rather than variation in the release of mediators from mast cells

in the skin. Four years later, the same group of investigators verified their initial findings with a computerized statistical method.² Lee et al.³ confirmed these findings and extended the observations to skin test responses to grass and house dust extracts. They suggested that false negative readings could result if skin tests were performed in the early morning office hours. However, this possibility has not been carefully examined. It was the purpose of our study to determine whether significant differences in the interpretation of skin test results could occur if testing was performed in the morning rather than in the afternoon.

MATERIAL AND METHODS

Forty ragweed-sensitive patients (20 adults and 20 children) attending the National Jewish Center for Immunology and Respiratory Medicine were recruited for the study. Informed consent was obtained. All patients had a wheal reaction of ≥ 3 mm on prick skin testing with 1:20 wt/vol of short ragweed extract. Patients with dermographism were excluded. The age range of the pediatric patients was 6 to 17 years with a mean $\pm$ SD of 13 ± 2.7 years. The corresponding ages of adult patients were 18 to 59 years (range) and 34 ± 2.7 years (mean $\pm$ SD). All patients refrained from taking antihistamines for at least 48 hours before testing. Each patient underwent two sessions of skin testing,

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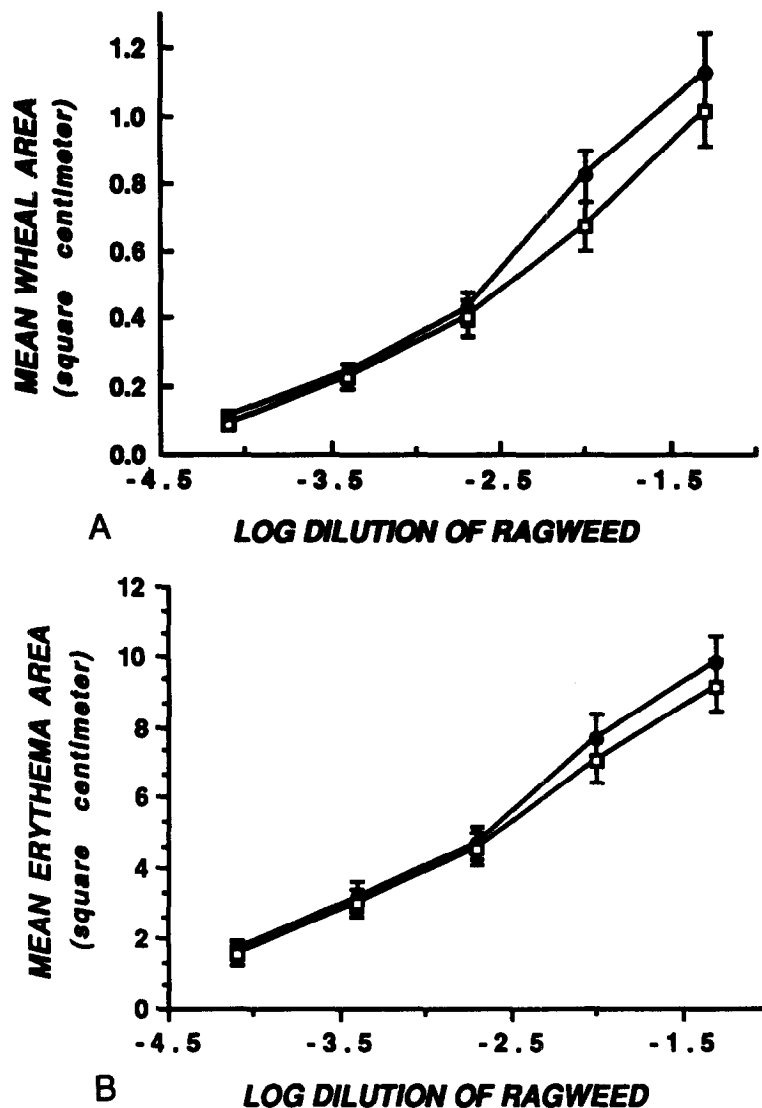


FIG. 1. Skin test areas (vertical axis) from all study subjects (20 adults and 20 children), obtained with short ragweed extracts at various dilutions (plotted as log dilutions on the horizontal axis), are presented for both (A) wheal and (B) erythema. Values obtained from the morning sessions and evening sessions are represented by filled circles and open squares, respectively. No significant difference was observed between morning and evening areas for either wheals or erythema at any dilutions tested.

one at 8 AM and the other at 4 PM, either on the same or consecutive days. Skin tests were performed on the back with five serial fivefold dilutions of both short ragweed extract (1:20 to 12,500 wt/vol, Greer Laboratory, Lenoir, N.C.) and histamine hydrochloride (10 to 0.016 mg/ml, Spectrum Chemicals, Gardena, Calif.), each performed in duplicate. Glycerosaline (50%) was used as a diluent and as a negative control. Each series of dilutions was performed in a column. The order of the dilutions within the column was alternated with one histamine and one ragweed column having the most concentrated solutions at the top and serial dilutions proceeding downward, and the other two columns in the reverse order. Therefore, no two concentrated extracts

were in adjacent columns. A template with sites 5 cm apart in all directions was used. At the second test session, this template was moved $\frac{3}{4}$ inch laterally to avoid use of identical test sites. The operator who performed all skin tests was blinded to the contents of the four series of vials until all the readings were completed. Skin test reading began after 10 minutes and was completed by 15 minutes. Reactions were graded 0 to 4+ according to a clinical scoring system⁴ and were outlined with a felt-tip pen. These outlines were then transferred with transparent tape to the patient's permanent record. The wheal-and-flare areas were measured by a computerized digitizing system (Bioquant II, R & M Biometrics, Inc., Nashville, Tenn.). Coefficient of variation

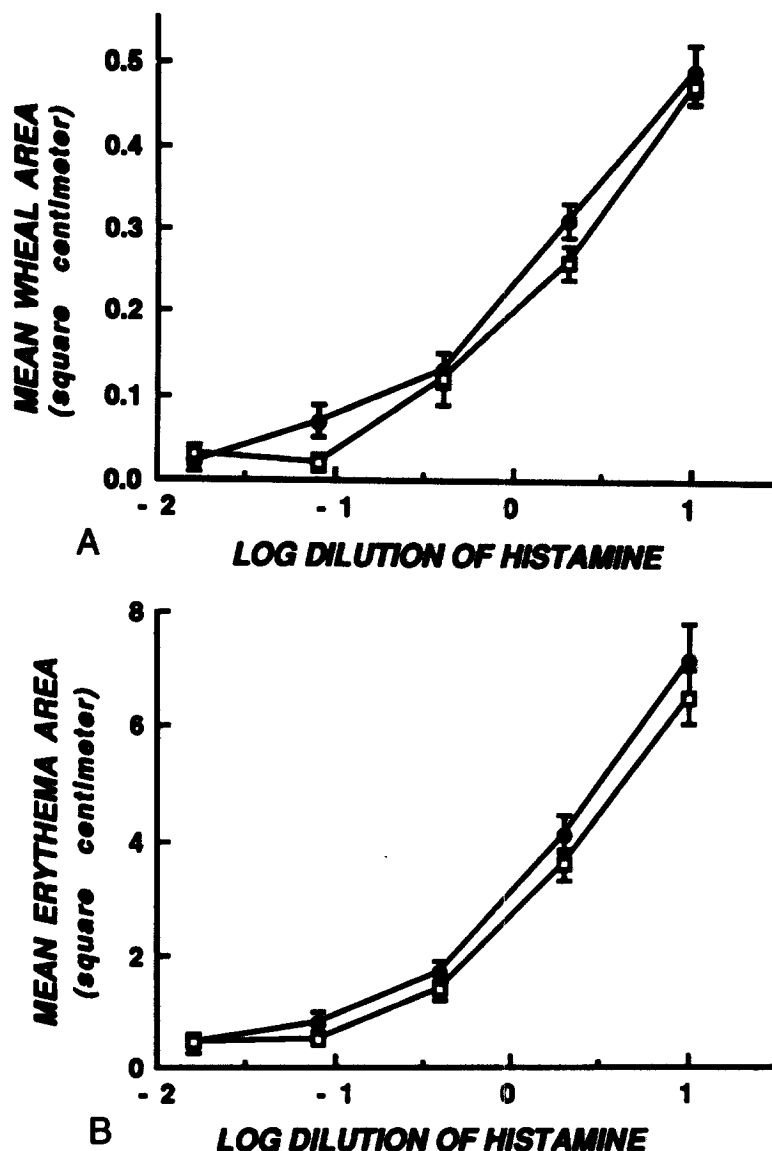


FIG. 2. Skin test areas (vertical axis) from all study subjects (20 adults and 20 children), obtained with histamine hydrochloride at various dilutions (plotted as log dilutions on the horizontal axis), are presented for both (A) wheal and (B) erythema. Legends are the same as in Figure 1. No significant difference was observed between morning and evening areas for either wheals or erythema at any dilutions tested.

of replicate measurements by this system was $\leq 5\%$. Both the duplicate skin tests were used for all calculations.

The wheal-and-flare areas, as well as clinical scores, were compared as outlined in statistical analysis.

Statistical analysis

Mean wheal-and-flare areas at various dilutions of ragweed and histamine from the morning test sessions were compared to the corresponding evening means by Student's paired *t* tests with Bonferroni's corrections (confidence limit of 95% for five comparisons). Wheal areas $< 0.07 \text{ cm}^2$ and flare areas $< 0.197 \text{ cm}^2$ were excluded from the analysis because of the high coefficient of variation in area mea-

surements of skin tests of these sizes.⁵ All areas (wheal and erythema from all dilutions) from each test session in each individual were later combined separately for ragweed and histamine and were converted into skin test index scores with a modification of a formula previously suggested by Voorhst.⁶

$$\text{Skin index score} = 2 \sqrt{\frac{(\text{Flare area})}{\pi}} + 1.85 + 5.75 \log 4 \sqrt{\frac{(\text{Wheal area})}{\pi}}$$

These indices for morning and evening values were directly

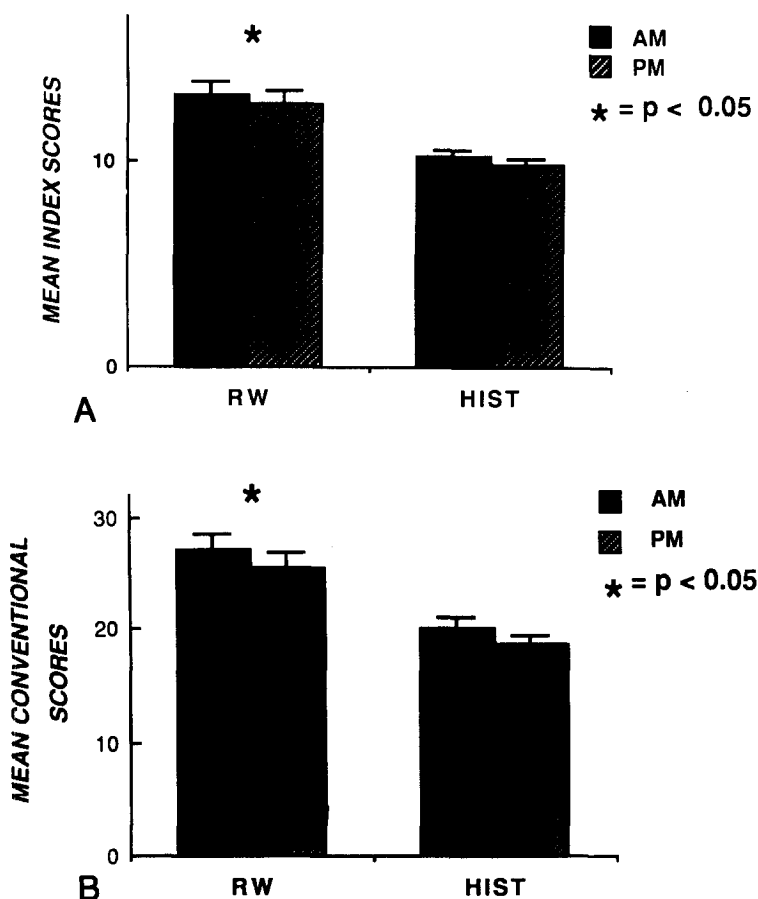


FIG. 3. Comparison between (A) morning versus evening mean index scores and (B) mean conventional scores from all patients studied. The only difference that was significantly different was in the ragweed group; Rw, short ragweed; Hist, histamine hydrochloride. Solid and hatched bars in each group represent morning and evening means, respectively.

compared by Student's paired *t* tests. Similarly, comparisons were conducted for the combined clinical scores. Correlation of skin index scores and conventional scores was examined by simple linear regression.

RESULTS

The comparisons between the mean morning and evening wheal and flare areas at various dilutions of ragweed and histamine from all patients are illustrated in Figs. 1 and 2. There was no statistically significant difference observed between the two means at any dilution of either skin test reagent. It is apparent from the data that at the commonly used dilutions for skin prick testing (1:20 wt/vol for ragweed and 10 mg/ml for histamine), no difference between morning versus evening wheal and erythema sizes was observed. When data were analyzed separately for adults and children, similar results were observed, except that the morning mean wheal areas at 1:100 wt/vol of ragweed and at 2 mg/ml of histamine were significantly larger than their corresponding evening

means in the pediatric age group. When data from each dose-response curve from Figs. 1 and 2 were interpolated with a simple linear regression equation, all coefficients of correlation were found to be ≥ 0.93 . Slopes from the morning curves were similar to their corresponding evening slopes, and as previously reported, slopes for erythema were greater than slopes for wheals.⁷ The wheal-and-flare areas in the morning and afternoon with ragweed and histamine were converted into skin index scores with a formula described above.⁶ As illustrated in Fig. 3, A, mean morning index scores were larger than mean evening scores for both ragweed and histamine. However, the difference was only significant ($p = 0.03$) for the ragweed group. Surprisingly, very similar findings were observed when the conventional scores were analyzed ($p = 0.03$ for ragweed and $p < 0.05$ for histamine, Fig. 3, B), suggesting that there is a good correlation between the two scoring systems. In fact, by linear regression (Fig. 4), these two systems were highly correlated ($r = 0.88$; $p < 0.001$).

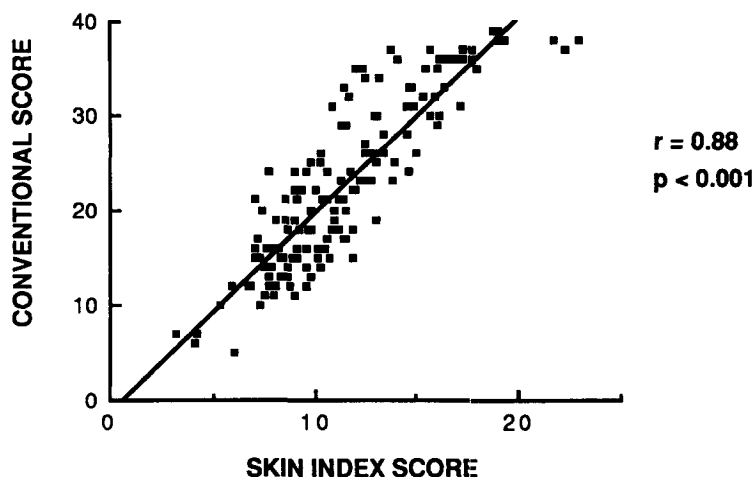


FIG. 4. Simple linear regression-correlation curve between conventional and skin index scores. The two scoring systems are highly correlated at $r = 0.877$ and $p < 0.001$.

There were 15 children who had the two skin test sessions performed consecutively. Seven children were tested first in the morning, whereas eight children were initially tested in the evening. No differences were observed between the morning and evening values in the two groups. Moreover, the differences between morning and evening values were not significantly different between the two groups. Thus, there was no evidence of a "refractory state" of the skin at the second test session, whether it was morning or evening.

DISCUSSION

In this study, no significant difference in skin reactivity to histamine or ragweed was observed when prick skin tests were performed at 8 AM and 4 PM. In fact, morning values were consistently larger than evening values for both histamine and ragweed; however, these differences were small and were not clinically and usually not statistically significant. In studies by Reinberg et al.¹ and Lee et al.,³ measurement of skin test results were expressed as percents of the 24-hour means and were then plotted on the response-time curve. Although there was clearly a trend toward increasing skin responses in the evening in these studies, it is difficult (1) to conceptualize the true differences and (2) to perform statistical comparisons between the true values obtained in the morning and evening. The reason that our results are different from results of previous studies is unclear. If, however, the underlying mechanism of the circadian variation of the cutaneous and the bronchial reactions to histamine are similar, it might be anticipated that the reactivity of the skin in the morning would be greater than, rather than less than, in the afternoon.^{8, 9} Our results fit well with this speculation. The smaller evening

values were not due to a refractory state of the skin because no differences were observed in values obtained from the two groups of children who had their first set of skin tests performed in the two differing sequences.

The value of computerized digitizing measurement in skin test research has been well established.^{10, 11} We eliminated smaller wheal areas ($<0.07 \text{ cm}^2$) and erythema areas (0.195 cm^2) from the statistical analysis because it has been demonstrated that the coefficient of variation for these smaller skin test reactions is quite large and, thereby, could confound the comparisons.⁵ To our surprise, the conventional scoring system, when it was carefully performed, correlated very well with the composite skin index score derived from the computerized measurement of wheal-and-flare areas. This reaffirmed the acceptability of the use of such a scoring system in clinical allergy practice.

In summary, we demonstrated that circadian variations in skin reactivity did not significantly alter the interpretation of the skin tests whether tests were performed and read in the early morning or in the late afternoon. Therefore, we conclude that it is not necessary in clinical allergy practice to consider the time of day that skin testing is performed in interpreting the results.

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