

# Salivary cortisol monitoring: Determination of reference values in healthy children and application in asthmatic children

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## ABSTRACT

*Venipuncture testing of adrenocortical function in asthmatic infants and young children receiving inhaled corticosteroids can raise cortisol levels and mask physiological responses. This study aimed to establish reference ranges for salivary cortisol levels and evaluate the safety and effects of jet-nebulized budesonide inhalation suspension (BIS) on salivary cortisol levels and patient outcomes in infants and young children with mild or persistent asthma. Reference salivary cortisol levels were determined in healthy children aged 6 months to 4 years old. A 12-week multicenter, randomized, parallel-group, open-label study was performed involving 53 age-matched asthmatic children who received either 0.5 mg/day of BIS or 40–60 mg/day of cromolyn sodium inhalation suspension (CIS) via compressor nebulizer. The effective measuring range of salivary cortisol concentration in asthmatic children was 0.12–3.00 micrograms/dL. The upper and lower limits of the reference range were 0.827 and 0.076 micrograms/dL, respectively. No significant difference was seen from baseline through week 12 in the CIS and BIS groups. BIS was safe in these patients, with no inhibitory effects on adrenocortical function. Salivary cortisol measurement offers a useful and accurate tool for testing adrenocortical function in infants and young children. Longer-term studies that incorporate testing of the hypothalamic–pituitary–adrenal axis are warranted to confirm our findings.*

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Asthma is the most common chronic disease among children and it begins at an early age.<sup>1</sup> Inhaled glucocorticosteroids are the preferred initial treatment to control it in infants and young children. However, long-term use requires regular testing of adrenocortical function for which plasma or serum cortisol levels are commonly used. However, venipuncture in this population is invasive and stressful enough to increase cortisol levels,<sup>2</sup> potentially masking physiological responses.

Correlations between the saliva and plasma concentrations of cortisol have been reported.<sup>3–6</sup> Therefore, determination of cortisol levels in saliva might offer an alternative method to assess adrenal function in infants and young children. The use of less invasive techniques might avoid the stress caused by invasive procedures, such as venipuncture, and enable accurate evaluation of physiological adrenocortical function.

The clinical application of saliva cortisol for assessing adrenocortical function in patients taking inhaled corticosteroids has been reported in adults and children with asthma,<sup>5–8</sup> but not in children <5 years of age—a population that lacks the precise timing of inhalation and adequate respiratory flow for effective use of pressurized metered-dose inhalers. In addition, no validated reference values have been established for salivary cortisol levels in healthy infants and young children, creating a need for reliable benchmarks.

Therefore, the primary objectives of the present study were (1) to establish reference values for salivary cortisol in healthy infants and children and (2) to compare the effects of treatment with budesonide inhalation suspension (BIS) or cromolyn sodium inhalation suspension (CIS) for 12 weeks on salivary cortisol levels in infants and young children.

## METHODS

### Study to Determine Salivary Cortisol Reference Values

**Subjects.** Reference salivary cortisol levels were determined in 57 healthy children, without asthma, aged from 6 months to 4 years old, whose height and weight were within the 3rd and 97th percentiles based on the 2000 Childhood Height and Growth Survey Report at the time of obtaining informed consent. The purpose and methods of the study were explained in detail to the parents or guardians, and written informed con-

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sent was obtained. The study protocol was approved by the local ethics committee.

**Saliva Collection.** Saliva samples were collected at subjects' homes by the parents or guardians using a Sorbette (Salimetrics LLC, State College, PA). Saliva samples were collected three times in the morning (at 8:00, 9:00, and 10:00 A.M.) on 5 separate days. Saliva was obtained by inserting a plastic stick fixed with an absorbent sponge made from natural materials into the end into the mouth (e.g., under the tongue). The plastic stick was rotated for ~1 minute to absorb saliva. After saliva collection, the absorbent sponge was placed in a tube, capped, and sealed. The samples were taken to the clinic on the same day.

**Measurement of Salivary Cortisol Concentrations.** Samples were centrifuged (3000 rpm, 5 minutes) and then stored at or below  $-20^{\circ}\text{C}$  until used. Cortisol levels in saliva samples were measured using a salivary cortisol EIA kit (Salimetrics LLC) at a central laboratory (SRL, Tokyo, Japan). The practical lower limit of detection was  $0.012\text{ }\mu\text{g/dL}$ , and intra- and interassay coefficients of variation (CVs) were 3.35–3.65% and 3.75–6.41%, respectively. According to the manufacturer's instructions, cross-reactivity of the assay with various steroid compounds was as follows: dexamethasone, 19.2%; prednisolone, 0.568%; corticosterone, 0.214%; 11-deoxycortisol, 0.156%; and cortisone, 0.130%.

To determine the impact of different cortisol concentrations on the reliability of the measured values, CVs were recalculated from 10 measurements of the standard solutions used to prepare the kit's calibration curve. These CVs were greater at test concentrations of  $0.037$  and  $0.012\text{ }\mu\text{g/dL}$  than at the other concentrations used ( $3.000\text{ }\mu\text{g/dL}$ , 1.2%;  $1.000\text{ }\mu\text{g/dL}$ , 1.3%;  $0.333\text{ }\mu\text{g/dL}$ , 1.1%;  $0.111\text{ }\mu\text{g/dL}$ , 2.0%;  $0.037\text{ }\mu\text{g/dL}$ , 4.3%; and  $0.012\text{ }\mu\text{g/dL}$ , 5.9%).

## Clinical Study

**Subjects.** Children aged 6 months to 5 years old with clinically diagnosed mild to moderate persistent asthma were included in the clinical study. The subjects included in the clinical study were independent of those included in the study to determine the salivary cortisol reference values. Exclusion criteria included a history of hypo- and hyperadrenocorticism, treatment with high doses of systemic glucocorticoids within the preceding 3 months, use of medication that could affect adrenal function within the preceding 3 months, fever with a temperature  $>38^{\circ}\text{C}$  at enrollment, and a history of hypersensitivity to study medication. Further use of systemic or inhaled steroids was not allowed during the study period.

**Study Design and Treatments.** This 12-week randomized, open-label, parallel-group study included a 1-day observation period during which subjects continued their current ICS medication and all other asthma medications were withdrawn. During this time, the parents and guardians received instructions from the physicians on how to collect salivary samples.

After the 1-day observation period and baseline assessment, eligible subjects were randomized to receive *via* nebulizer either BIS (Pulmicort Respules; AstraZeneca, Lund, Sweden) at  $0.5\text{ mg}$  once daily or  $0.25\text{ mg}$  twice daily (BIS group) or CIS (Intal; Astellas Pharma, Tokyo, Japan) at  $20\text{ mg}$  twice or three times daily (CIS group). The inhalation device for both medications was a PARI TurboBOY N compressor with a PARI LC Plus nebulizer (PARI, Stanberg, Germany).

After the baseline/randomization visit, the subjects and their caregivers were asked to return to the clinic at the end of weeks 2, 4, 6, 8, 10, and 12. Salivary samples were collected in the morning before the clinic visits at baseline and at weeks 2, 4, 8, and 12.

The study protocol was approved by the hospital's external ethics committee before enrollment, and the research was performed in accordance with the Declaration of Helsinki. Parents or legal guardians provided written informed consent.

**Saliva Collection.** Saliva samples were collected essentially as described previously. Samples were collected on the morning of visits at baseline and at weeks 2, 4, 8, and 12. Samples were collected between 6:30 and 8:30 A.M. and the patients were not permitted to eat breakfast until after sample collection had been completed.

**Patient Outcomes.** Patient outcomes were determined using a daily diary in which the patients' caregivers recorded daytime and nighttime asthma symptoms, cough, nighttime awakening, and the time of salivary sample collection. Asthma symptoms were evaluated by parents or guardians on a 10-point scale, where 0 = no symptoms and 9 = severe symptoms. Cough scores were evaluated on a 2-point scale, where 0 = no cough and 1 = cough. Nighttime awakening was assessed on a 4-point scale where 0 = a good night's sleep and 3 = could not sleep at all because of difficulty breathing.

**Safety Assessments.** Adverse events and changes in salivary cortisol from baseline were used to evaluate safety. Adverse events were recorded by the parent or guardian and were reviewed by the physician at each visit.

## Statistical Analyses

**Reference Values and Validation.** Because logarithmic transformation of the raw values showed a near-nor-

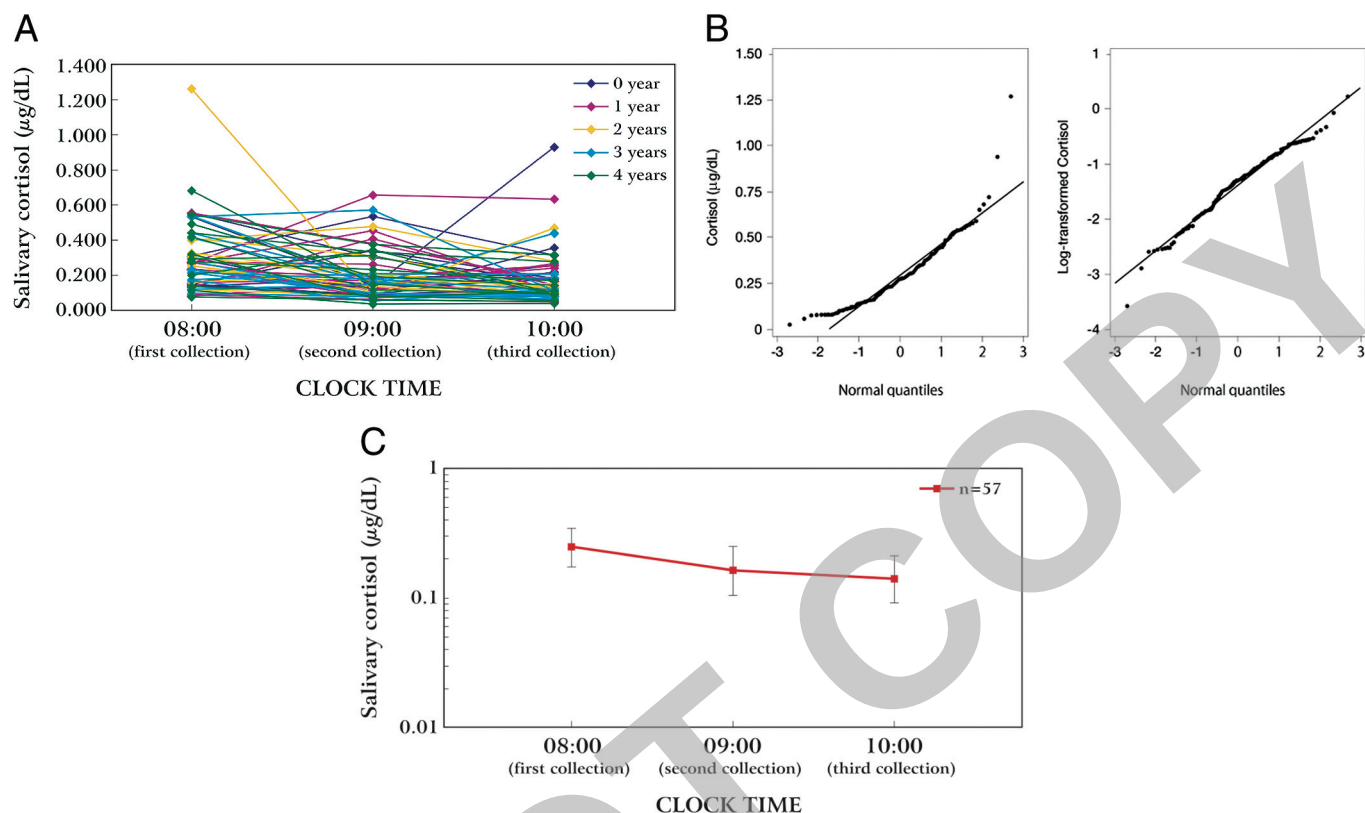


**Figure 1.** (A) Salivary cortisol levels in individual patients. (B) Q-Q plots for raw and log-transformed cortisol data. Logarithmic transformation of actual values for all three measurements at 8:00 A.M. showed a more near-normal distribution. (C) Log-transformed salivary cortisol levels showing the linear decrease in levels between 8:00 and 10:00 A.M.

mal distribution, we performed mixed-effect model analysis using logarithmic transformed values for diurnal variation and interday variation in salivary cortisol levels. The mixed-effect model for repeated measurement data was used to analyze both diurnal and interday variations. The model for diurnal variation included fixed effects for time, age group, and an interaction between time and age, with repeated measurements taken into consideration. The model for interday variation included fixed effects for sample day, age group, and an interaction between sample day and age. Compound symmetry was assumed as a working covariance structure. Two-sided values of  $p < 0.05$  were considered statistically significant.

**Primary and Secondary Outcomes in the Clinical Study.** The primary outcome was the change in mean salivary cortisol from baseline to week 12. The secondary outcomes were changes in mean salivary cortisol between baseline and 2, 4, and 8 weeks. Nonnormally distributed variables were logarithmically transformed. The changes from baseline were compared between the BIS and CIS groups using two-sample  $t$ -tests. A value of  $p \leq 0.05$  was considered statistically significant.

## RESULTS

### Reference Values and Validation

Diurnal variations exist in salivary cortisol levels in healthy children  $>1$  year of age, as in adults.<sup>9</sup> Indeed, we found a significant negative linear trend in salivary cortisol levels between 8:00 and 10:00 A.M. that was apparent on an individual basis (Fig. 1 A). The actual concentrations of salivary cortisol in all subjects on the 1st day of evaluation were highest at 8:00 A.M. (mean  $\pm$  SD,  $0.295 \pm 0.196$  µg/dL) and, subsequently, decreased at 9:00 A.M. ( $0.202 \pm 0.141$  µg/dL) and 10:00 A.M. ( $0.178 \pm 0.152$  µg/dL). Because logarithmic transformation of the actual concentrations showed a nearly normal distribution based on Q-Q plots (Fig. 1 B), the logarithmic transformed value was plotted against clock time. This analysis revealed a decreasing linear trend in salivary cortisol level across sample times ( $p < 0.0001$ ; linear trend test using a linear contrast with coefficients  $[-1, 0, 1]$  based on mixed-effect model analysis), and hence showed the presence of diurnal variation (Fig. 1 C).

No significant differences in salivary cortisol level were seen among age groups ( $p = 0.6674$ ), but a significant interaction of sample day with age was ob-

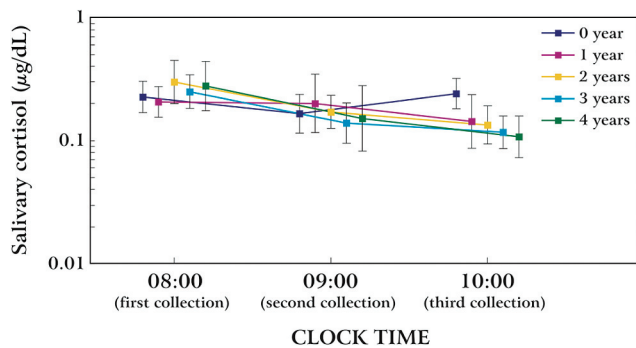


**Figure 2.** Diurnal changes in salivary cortisol are apparent in children aged 2–4 years, but not in children aged 0–2 years. At 2–4 years of age, a significant decreasing linear trend was seen from 8:00 to 10:00 A.M. (0 years,  $p = 0.7690$ ; 1 year,  $p = 0.0755$ ; 2 years,  $p = 0.0005$ ; 3 years,  $p = 0.0004$ ; 4 years,  $p < .0001$ ; linear trend tests using linear contrasts applied with coefficients  $[-1, 0, 1]$  based on mixed-effects model analysis).

served ( $p = 0.0206$ ; Fig. 2). The results of this analysis suggest that the day of sample collection and age together influence salivary cortisol levels, whereas neither parameter examined in isolation significantly influences cortisol levels. Patterns of change were evaluated within different age groups. A significant decreasing linear trend was seen from 8:00 to 10:00 A.M. in the 2-, 3-, and 4-year-old groups, but not in the 0- and 1-year-old groups (0 years,  $p = 0.7690$ ; 1 year,  $p = 0.0755$ ; 2 years,  $p = 0.0005$ ; 3 years,  $p = 0.0004$ ; 4 years,  $p < 0.0001$ ; linear trend tests using linear contrasts applied with coefficients  $[-1, 0, 1]$  based on mixed-effect model analysis). Meanwhile, salivary cortisol levels within three samples collected at 8:00 A.M. at 4- to 8-day intervals showed no significant interday variation ( $p = 0.3570$ ; Fig. 3 A). Furthermore, there were no differences among age groups ( $p = 0.7577$ ) and there was no interaction between sample day and age ( $p = 0.2888$ ; Fig. 3 B).

Reference values for changes by age were calculated from all of these samples at 8:00 A.M. (57 subjects, 171 samples). The mean  $\pm$  SD (after logarithmic transformation) of all these sample values was  $0.251 \pm 1.815$ . The  $-2$  SD and  $+2$  SD values were  $0.076 \mu\text{g/dL}$  and  $0.827 \mu\text{g/dL}$ , respectively. For interday variation data at 8:00 A.M., after logarithmic transformation, interindividual CVs for the first, second, and third measurements were 42, 47, and 40%, respectively (mean 43%). Intraindividual CV for samples obtained at 08:00 A.M. was 33%.

## Clinical Study

**Patients.** The randomized controlled phase of the study was conducted at eight Japanese sites. The disposition of subjects is summarized in Fig. 4. In total, 83% (44/53) of the subjects randomized to treatment

with either BIS or CIS completed the study—77.78% (21/27) in the BIS group and 88.4% (23/26) in the CIS group. The primary reasons for treatment discontinuation were the use of systemic steroids within the preceding 3 months and treatment discontinuation (7.5% overall).

Demographic and baseline clinical characteristics were similar between the two treatment groups (Table 1). Most of the subjects were between 1 and 2 years of age (65%) and the mean ages of subjects in the BIS and CIS groups were  $25.7 \pm 14.8$  months and  $26.7 \pm 16.1$  months, respectively. The mean duration since diagnosis of asthma was  $11.2 \pm 10.4$  months and  $7.9 \pm 6.6$  months, respectively. Just over one-half of the patients in both groups had moderately severe asthma (BIS, 57%; CIS, 56.5%).

**Analysis of Salivary Cortisol Levels.** In total, 44 patients (194 samples) were analyzed, including 21 patients (89 samples) in the BIS group and 23 patients (105 samples) in the CIS group. Reasons for excluding samples are presented in Table 2. Figure 5 shows the absolute salivary cortisol concentrations measured at weeks 0, 2, 4, 8, and 12. After logarithmic transformation, salivary concentrations did not differ significantly among the treatment groups. The effective range for measuring salivary cortisol concentrations was  $0.12\text{--}3.000 \mu\text{g/dL}$ .

**Patient Outcomes.** Demographic features and asthma history were similar, with no significant differences between groups at baseline for asthma symptoms and cough scores (Table 1). As shown in Table 3, nighttime asthma symptom scores improved in both groups, with marked reductions occurring within 1–2 weeks. Table 3 also shows the improvements from baseline in daytime asthma symptom scores, cough scores, and nighttime awakening during the 12-week study. The two groups were similar for all parameters.

**Safety.** No drug-related adverse reactions were recorded in any group in the present study.

## DISCUSSION

The serum cortisol level is well known to show diurnal variation, with the highest levels in the early morning and lowest around midnight. The same diurnal variation has been reported for salivary cortisol levels in adults.<sup>10</sup> Hence, it is important to establish reference values in children to apply them in clinical studies. We found that the salivary cortisol levels were highest at 8:00 A.M. and then decreased linearly at 9:00 and 10:00 A.M. in most subjects. This result shows that short-term sampling also reflects the diurnal variation in serum cortisol levels.

Because the analysis by age group did not show these changes in 0- to 1-year-old children, a diurnal

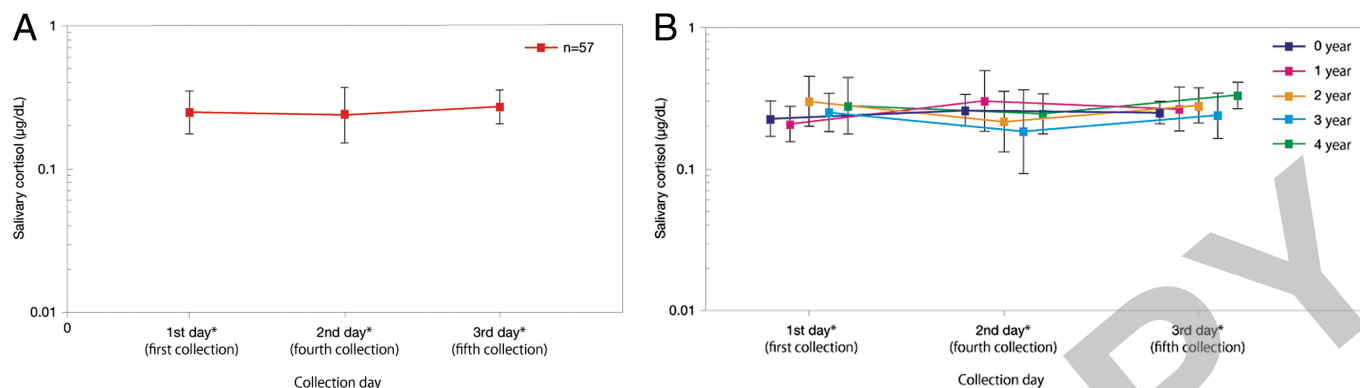


**Figure 3.** Interday variation in salivary cortisol levels (log transformation) (A) for the entire study population and (B) for each age group. Salivary cortisol levels from 8:00 A.M. samples collected three times, at 4- to 8-day intervals. Statistical analyses showed no significant interday variation ( $p = 0.3570$ ), differences among age groups ( $p = 0.7577$ ), or an interaction between age and sample day ( $p = 0.2888$ ).

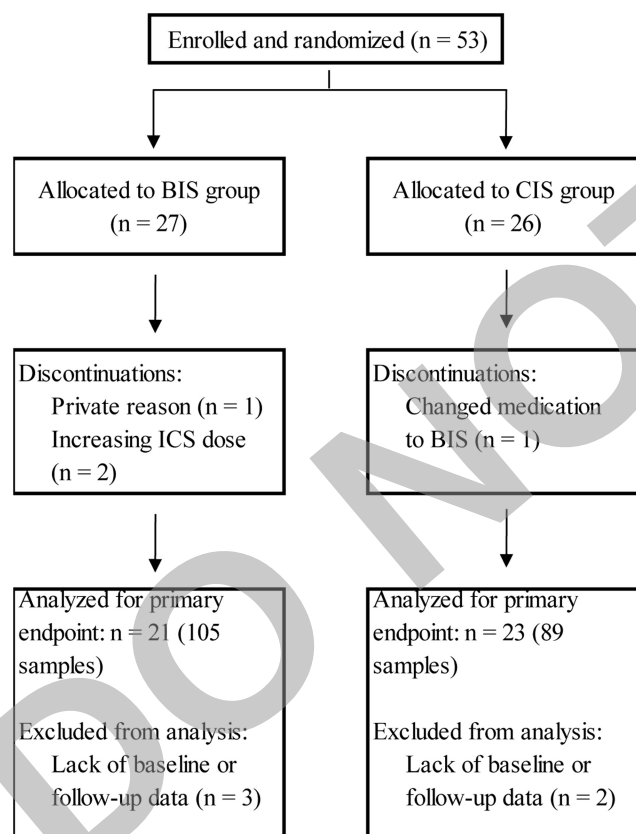


**Figure 4.** Subject disposition. Budesonide inhalation suspension (BIS) at 0.5 mg once daily or 0.25 mg twice daily; cromolyn sodium inhalation suspension (CIS) at 20 mg two or three times daily; jet nebulizer;  $n = 44$ .

rhythm may not be established in these age groups. However, Price *et al.* showed circadian rhythms by 3 months of age in saliva samples collected at 8:00 A.M. and 12:00, 4:00, and 8:00 P.M.<sup>9</sup>

We also performed a 12-week study of jet-nebulized budesonide in infants and young children in which the primary outcome was salivary cortisol levels. We evaluated the reliability of the findings compared with

validated reference values of salivary cortisol levels in healthy, age-matched controls. Very few randomized controlled trials<sup>11–14</sup> have investigated the adrenocortical effects of inhaled steroids in infants and, to our knowledge, none have compared outcomes against validated reference ranges of salivary cortisol levels in healthy infants. These two factors make this study unique.

Data from this study support the hypothesis that inhalation of 0.5 mg of BIS has no inhibitory effect on the adrenocortical function of infants with asthma. These findings are consistent with those of other studies.<sup>15</sup> Previous reports have indicated no significant effects of budesonide on adrenal function in patients 6–12 months of age with mild to moderate persistent asthma or recurrent wheeze.<sup>16</sup> Indeed, extensive safety data for inhaled budesonide (administered as nebulized suspension or *via* dry-powder inhalers) are available in infants and children aged  $\geq 6$  months.<sup>17</sup>

Our outcomes suggest that measurement of salivary cortisol levels is an easy and accurate means to test adrenocortical function in infants and young children and a viable alternative to testing of plasma cortisol levels. However, the mechanism of cortisol secretion into saliva is unclear. Possibly because of differences in saliva characteristics by salivary gland type, wider variations in salivary cortisol levels compared with blood and urinary cortisol levels have been reported.<sup>18</sup> Importantly, no drug-related adverse reactions were recorded in any group in the present study.

We also collected saliva samples from two adults with congenital adrenal hyperplasia. Saliva cortisol levels were 0.036 and 0.018  $\mu\text{g}/\text{dL}$  in one patient, and 0.028  $\mu\text{g}/\text{dL}$  in the other patient. Both patients were under treatment with hydrocortisone (after breakfast) and dexamethasone (after dinner). The ability to measure salivary cortisol in these patients provides further

**Table 1 Baseline characteristics of subjects included in the randomized controlled trial**

| Characteristic                      | BIS ( <i>n</i> = 21) | CIS ( <i>n</i> = 23) |
|-------------------------------------|----------------------|----------------------|
| Age                                 |                      |                      |
| 6 mo to <1 yr                       | 2                    | 6                    |
| 1 to <2 yr                          | 10                   | 5                    |
| 2 to <3 yr                          | 3                    | 5                    |
| 3 to <4 yr                          | 4                    | 4                    |
| 4 to <5 yr                          | 2                    | 3                    |
| Age (mo), mean $\pm$ SD             | 25.7 $\pm$ 14.8      | 26.7 $\pm$ 16.1      |
| Gender                              |                      |                      |
| Male                                | 13                   | 11                   |
| Female                              | 8                    | 12                   |
| Asthma duration                     |                      |                      |
| <6 mo                               | 6                    | 12                   |
| 6 mo to <1 yr                       | 8                    | 6                    |
| 1 to <2 yr                          | 5                    | 2                    |
| 2 to <3 yr                          | 1                    | 2                    |
| 3 to <4 yr                          | 1                    | 0                    |
| 4 to <5 yr                          | 0                    | 0                    |
| Unknown                             | 0                    | 1                    |
| Asthma duration (mo), mean $\pm$ SD | 11.2 $\pm$ 10.4      | 7.9 $\pm$ 6.6        |
| Severity                            |                      |                      |
| Mild                                | 9                    | 10                   |
| Moderate                            | 12                   | 13                   |
| Asthma symptom scores (mean)        | ( <i>n</i> = 20)     | ( <i>n</i> = 23)     |
| Daytime symptoms (0–9)              | 0.45                 | 1.04                 |
| Nighttime symptoms (0–9)            | 1.35                 | 1.43                 |
| All day (0–18)                      | 1.80                 | 2.48                 |
| Cough (mean)                        | ( <i>n</i> = 20)     | ( <i>n</i> = 23)     |
| Daytime cough                       | 0.40                 | 0.57                 |
| Nighttime cough                     | 0.50                 | 0.62                 |
| All day                             | 0.90                 | 1.22                 |

BIS = budesonide inhalation suspension; CIS = cromolyn sodium inhalation suspension.

confirmation that this is a tool that could be applied to a range of disease settings to monitor adrenocortical function. Additional studies are needed to confirm these findings in a larger cohort of patients and determine the validity of measuring salivary cortisol in other related diseases.

This study is subject to other limitations, including its short duration and lack of testing of cross-reactivity of the assay against inhaled corticosteroids. We collected salivary samples only in the morning, but doing so in the evening might have allowed us to detect diurnal variations. Other possibilities are that the sensitivity and specificity of methods for measuring sali-

**Table 2 Summary of saliva samples used for analysis of salivary cortisol in the randomized controlled trial**

|                                                                                      | Saliva samples, <i>n</i> (no. of patients) |
|--------------------------------------------------------------------------------------|--------------------------------------------|
| Samples collected from all patients included in the study                            |                                            |
| Total no. of samples collected                                                       | 253 (53)                                   |
| Samples excluded because the patient withdrew before randomization                   | 2 (1)                                      |
| Samples excluded because the patient committed a protocol deviation                  | 10 (2)                                     |
| Samples from a patient who discontinued in the early phase of the study              | 5 (2)                                      |
| Samples from patients included in the study                                          | 236 (49)                                   |
| Patient withdrew during the study                                                    | 4 (4)                                      |
| Determination was impossible because of insufficient sample                          | 10 (8)                                     |
| Concentration below the lower limit of determination                                 | 2 (1)                                      |
| Sample not collected within the allotted time, or the time of collection was unknown | 10 (7)                                     |
| Excluded because of fever                                                            | 3 (3)                                      |
| Absence of previous value or previous value only                                     | 13 (5)                                     |
| Total number of samples used in the final analysis                                   | 194 (44)                                   |

vary cortisol are too low to make them an adequate alternative to serum cortisol measurement,<sup>19</sup> or that our study was too underpowered to detect an effect on the hypothalamic—pituitary—adrenal (HPA) axis not readily apparent from early morning saliva cortisol. Prior studies have indicated that urinary free cortisol and area under the curve of serum cortisol, especially nocturnal ones, show HPA suppression not readily apparent from early morning serum cortisol measurements.<sup>20</sup> In addition, we did not perform dynamic tests of the HPA axis. It is possible that measurement of cortisol at a single time (*e.g.*, 8:00 A.M.) may miss some patients with suppression of the HPA axis. Thus, future studies should also incorporate adrenocorticotrophic hormone stimulation tests or insulin tolerance tests to confirm the validity of our approach.

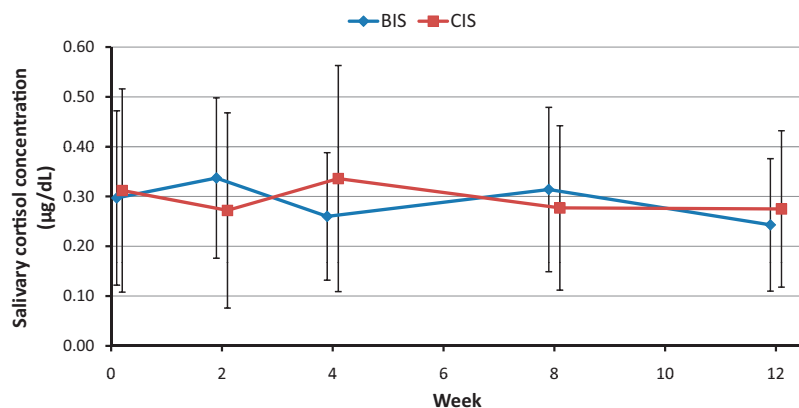


**Figure 5.** Salivary cortisol concentrations in patients taking budesonide inhalation suspension (BIS) or cromolyn sodium inhalation suspension (CIS). Values are means  $\pm$  SD.

**Table 3** Changes in asthma scores from baseline

|                                | Week | BIS      |      | CIS      |       |
|--------------------------------|------|----------|------|----------|-------|
|                                |      | <i>n</i> | Mean | <i>n</i> | Mean  |
| Daytime asthma symptom score   | 0    | 22       | 2.86 | 23       | 6.39  |
|                                | 2    | 21       | 1.43 | 23       | 2.74  |
|                                | 4    | 20       | 1.05 | 23       | 2.22  |
|                                | 8    | 22       | 0.96 | 22       | 0.68  |
|                                | 12   | 20       | 0.45 | 22       | 0.82  |
| Nighttime asthma symptom score | 0    | 22       | 8.59 | 23       | 9.13  |
|                                | 2    | 21       | 1.86 | 23       | 3.78  |
|                                | 4    | 20       | 1.05 | 23       | 2.48  |
|                                | 8    | 22       | 0.96 | 22       | 2.18  |
|                                | 12   | 20       | 1.05 | 22       | 1.77  |
| Daytime cough                  | 0    | 22       | 2.86 | 23       | 3.65  |
|                                | 2    | 21       | 3.29 | 23       | 2.70  |
|                                | 4    | 20       | 3.20 | 23       | 2.35  |
|                                | 8    | 22       | 1.82 | 22       | 1.36  |
|                                | 12   | 20       | 1.60 | 22       | 1.32  |
| Nighttime awakening            | 1    | 22       | 7.32 | 23       | 10.96 |
|                                | 2    | 21       | 4.00 | 23       | 4.87  |
|                                | 4    | 20       | 0.60 | 23       | 0.87  |
|                                | 8    | 22       | 0.41 | 22       | 0.64  |
|                                | 12   | 20       | 0.35 | 22       | 0.18  |

BIS = budesonide inhalation suspension; CIS = cromolyn sodium inhalation suspension.

In the present study, salivary cortisol levels in healthy children showed some evidence of diurnal variation, which may mirror the variation seen in serum cortisol levels. Because salivary cortisol levels showed no inter-day variations, the reference range was established at 8:00 A.M. Clinical studies that use this method should collect samples at around 8:00 A.M. (or at the time when cortisol concentrations should be close to maximal) to avoid any influence of the diurnal variation.

## CONCLUSIONS

Here, we have established reference ranges for salivary cortisol in healthy children and shown diurnal variations in these levels, meaning saliva collection

should be performed at specific times, preferably at around 8:00 A.M. Data from this 12-week study support the hypothesis that twice-daily inhalation of 0.5 mg of BIS does not suppress the adrenocortical function of infants with asthma, based on their saliva cortisol levels compared with validated reference ranges for healthy infants and adults. These findings suggest that salivary cortisol levels could be used to evaluate adrenal function in asthmatic infants and children who use inhaled corticosteroids, although this possibility must be confirmed in studies that also test HPA activity, using adrenocorticotrophic hormone stimulation or insulin tolerance tests. The outcomes of this study warrant additional longer-term studies.

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